Preclinical Drug Development

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Drug Development

- **Drug discovery & screening**
- **Preclinical development**
- **Animal scale up**
- **Phase I studies**
- **Phase II studies**
- **Phase III studies**

Goals of Preclinical Development

- **Transition between identification of a novel, promising compound and the initiation of human clinical trials**
- **Examples from anticancer drug development**
- **Specifics of the National Cancer Institute drug development program**

Components of Preclinical Drug Development

1. **In vitro studies: Cell lines, cell-free systems (drug screening)**
2. **Drug supply & manufacturing**
3. **Drug formulation**
4. **In vivo studies: Animal models and proof of principle**
   - **Efficacy**
   - **Toxicity**

In Vitro Study Goals: Define the Drug's Pharmacology

- **Molecular mechanism of action and specific drug targets**
- **Molecular pharmacology**
- **Determinants of response**
- **Intracellular pharmacodynamics**
- **Mechanisms of drug resistance**

In Vitro Study Systems

- **Cell-free assay for specific molecular effects**
  - **Enzyme inhibition, receptor blockade, etc.**
- **Yeast-based screening in genetically defined target**
- **Mammalian cell lines: (murine, human, etc.)**

What specific pharmacologic drug properties may be defined during preclinical in vitro testing of new anticancer agents?

Preclinical Pharmacology
In Vitro Studies of Cancer Agents (1)

- **Define anticancer effects**
  - **Growth inhibition, differentiation, apoptosis, etc**
- **Impact on defined biochemical and molecular pathways**
  - **RNA, DNA and protein biosynthesis, signaling kinases, etc**
- **Spectrum of antitumor activity**
  - **Human tumor cell lines**

Preclinical Pharmacology
In Vitro Studies of Cancer Agents (2)

- **Cellular uptake and membrane transport**
  - **MDR, MRP, etc**
- **Mechanisms of resistance**
- **In vitro drug metabolism**
  - **P450 isoenzymes**

Components of Preclinical Drug Development

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Drug Supply and Formulation

- **Drug supply: bulk chemical synthesis, natural product isolation, etc.**
- **Good Manufacturing Practice (GMP) guidelines for pharmaceutical product manufacturing**
- **Formulation for clinical delivery of drug: vehicles for intravenous or other routes of administration**

Drug Supply Issues

- **Paclitaxel source from the bark and wood of the Pacific Yew tree**
- **Early drug supply limited the amount available for initial clinical trials**
- **Newer semisynthetic production from the needles of the Yew tree (renewable)**

Drug Formulation Issues

- **Poor water solubility of natural products**
- **Paclitaxel formulation in cremophore EL (increased toxicity?)**
- **Camptothecin derivatives formulated in a dimethylacetamide, polyethylene glycol and phosphoric acid vehicle**
  - **Later formulated as a lipid colloidal dispersion**

Components of Preclinical Drug Development

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   - **Efficacy**
   - **Toxicity**

In Vivo Study Goals: Animal Models

- Efficacy: Proof of therapeutic principle
- Toxicology: Toxicity profile
- Practical Issues:
  - Animal pharmacokinetics and pharmacodynamics
  - Starting dose and schedule for clinical trials

Animal Models
Proof of Principle

- **Animal screening is too expensive for routine use**
- **Efficacy in animal models of specific disease states occurs after in vitro studies**
- **Evaluation of therapeutic index**
  - **Toxicity versus efficacy**

Ideal Animal Model

- Validity
- Selectivity
- Predictability
- Reproducibility

"There is no perfect tumor model"

Animal Models in Cancer

- **Spontaneous tumors**
  - **Idiopathic**
  - **Carcinogen-induced**
  - **Transgenic/gene knockout animals: p53, RB, etc**
- **Transplanted tumors**
  - **Animal tumors: Lewis lung, S180 sarcoma, etc**
  - **Human tumor xenografts: human tumor lines implanted in immunodeficient mice (current NCI standard in vivo efficacy testing system)**
  - **Human tumors growing in vivo in implantable hollow fibers**

Human Tumor Xenografts

- **Athymic "nude"mice developed in 1960's**
- **Mutation in *nu* gene on chromosome 11**
- **Phenotype: retarded growth, low fertility, no fur, immunocompromised**
  - **Lack thymus gland, T-cell immunity**
- **First human tumor xenograft of colon adenocarcinoma by Rygaard & Poulson, 1969**

Murine Xenograft Sites

- **Subcutaneous tumor (NCI method of choice) with IP drug administration**
- **Intraperitoneal**
- **Intracranial**
- **Intrasplenic**
- **Renal subcapsule**
- **Site-specific (orthotopic) organ inoculation**

Xenograft Study Endpoints

- **Toxicity Endpoints:**
  - **Drug related death**
  - **Net animal weight loss**
- **Efficacy Endpoints**
  - **Clonogenic assay**
  - **Tumor growth assay (corrected for tumor doubling time)**
  - **Treated/control survival ratio**
  - **Tumor weight change**

Xenograft Tumor Weight Change

- **Tumor weight change ratio (used by the NCI in xenograft evaluation)**
- **Defined as: treated/control x 100%**
- **Tumor weight in mg = $(a \times b^2)/2$**
  - **a = tumor length**
  - **b = tumor width**
- **T/C < 40-50% is considered significant**

Xenograft Advantages

- **Many different human tumor cell lines transplantable**
- **Wide representation of most human solid tumors**
- **Allows for evaluation of therapeutic index**
- **Good correlation with drug regimens active in human lung, colon, breast, and melanoma cancers**

Xenograft Disadvantages

- **Brain tumors difficult to model**
- **Different biological behavior, metastases rare**
  - **Survival not an ideal endpoint: death from bulk of tumor, not invasion**
- **Shorter doubling times than original growth in human**
- **Less necrosis, better blood supply**
- **Difficult to maintain animals due to infection risks**

Other Animal Models

- **Orthotopic animal models: Tumor cell implantation in target organ**
  - **Metastatic disease models**
- **Transgenic Animal Models**
  - **P53 or other tumor suppressor gene knockout animals**
  - **Endogenous tumor cell development**

In Vivo Hollow Fiber Assay

- **In vivo screening tool implemented in 1995 by NCI**
- **12 human tumor cell lines (lung, breast, colon, melanoma, ovary, and glioma**
- **Cells suspended into hollow polyvinylidene fluoride fibers implanted IP and SC in lab mice**
- **After in vivo drug treatment, fibers are removed and analyzed in vitro**
- **Antitumor (growth inhibitory) activity assessed**

Animal Models
PK/PD Studies

- **Analytic assay development and testing**
- **Preclinical PK/PD relationships**
- **Initial drug formulation testing**
- **Testing of different schedules and routes of administration**

Preclinical Toxicology Goals

- **Estimate a "safe" starting dose for phase I studies**
- **Determine the toxicity profile for acute and chronic administration**
- **NCI guidelines recommend single dose and multidose toxicity in two species (one non-rodent)**
- **FDA guidelines are 1/10 the LD_{10} in mice**

Preclinical Toxicology Background: Pre-1980's

- **NCI used dogs and monkeys for lethal and non-lethal dose determination**
- **Chronic toxicity testing in dogs**
- **Starting clinical dose 1/3 lowest toxic dose in the most sensitive animal model, monkey or dog**

NCI Toxicology Requirements (Div. Cancer Treatment, 1980)

- **Murine single dose and multidose (daily x 5) to determine the LD_{10}, LD_{50}, and LD_{90}.**
- **LD_{10} converted to mg/m^2 is defined as the mouse equivalent LD_{10}($MELD_{10}$)**
- **1/10 the $MELD_{10}$ given to beagle dogs**
  - **If no toxicity, dose is escalated until minimal reversible toxicity is seen, defined as toxic dose low (TDL)**
  - **TDL is the lowest dose that produces drug induced pathologic changes in hematologic, chemical, clinical or morphologic parameters**
  - **Double the TDL produces no lethality**
- **Human equivalent of 1/3 the TDL in dogs is the recommended phase I starting dose**

Species Dose Conversion

- **Dog $MELD_{10}$ (mg/m^2) = (Km dog/Km mouse) x LD_{10} mouse (mg/m^2)**
  - **Where Km is the surface area to weight ratio**
  - **Km dog = 20, Km mouse = 3.0 and adult human Km = 37**

EORTC Toxicology Guidelines

- **Rodent only toxicology for anticancer agents adopted in 1980, revised in 1992**
- **Full studies in mice and limited studies in rats**
- **Use 1/10 the mouse LD_{10} as the clinical Phase I starting dose**

Anticancer Drug Development at the National Cancer Institute

History of the NCI Drug Development Programs

- **1955: Cancer Chemotherapy National Service Center screening initiated (NSC#)**
- **1975-1989: In vivo screening using P388 and L1210 murine leukemias**
- **1985-1990: Disease-oriented screening using 60 human tumor cell lines**

History of the NCI Drug Development Programs

- **1998 and beyond: molecular target based screening using the 60 cell line screen**
- **Yeast based genetically defined screening**
- **Drug development at the NCI is overseen by the Developmental Therapeutics Program (DTP) led by Dr. Ed Sausville**
  - **Current guidelines at NCI DTP website at http://dtp.nci.gov**

Three Cell Line In Vitro Pre-Screen

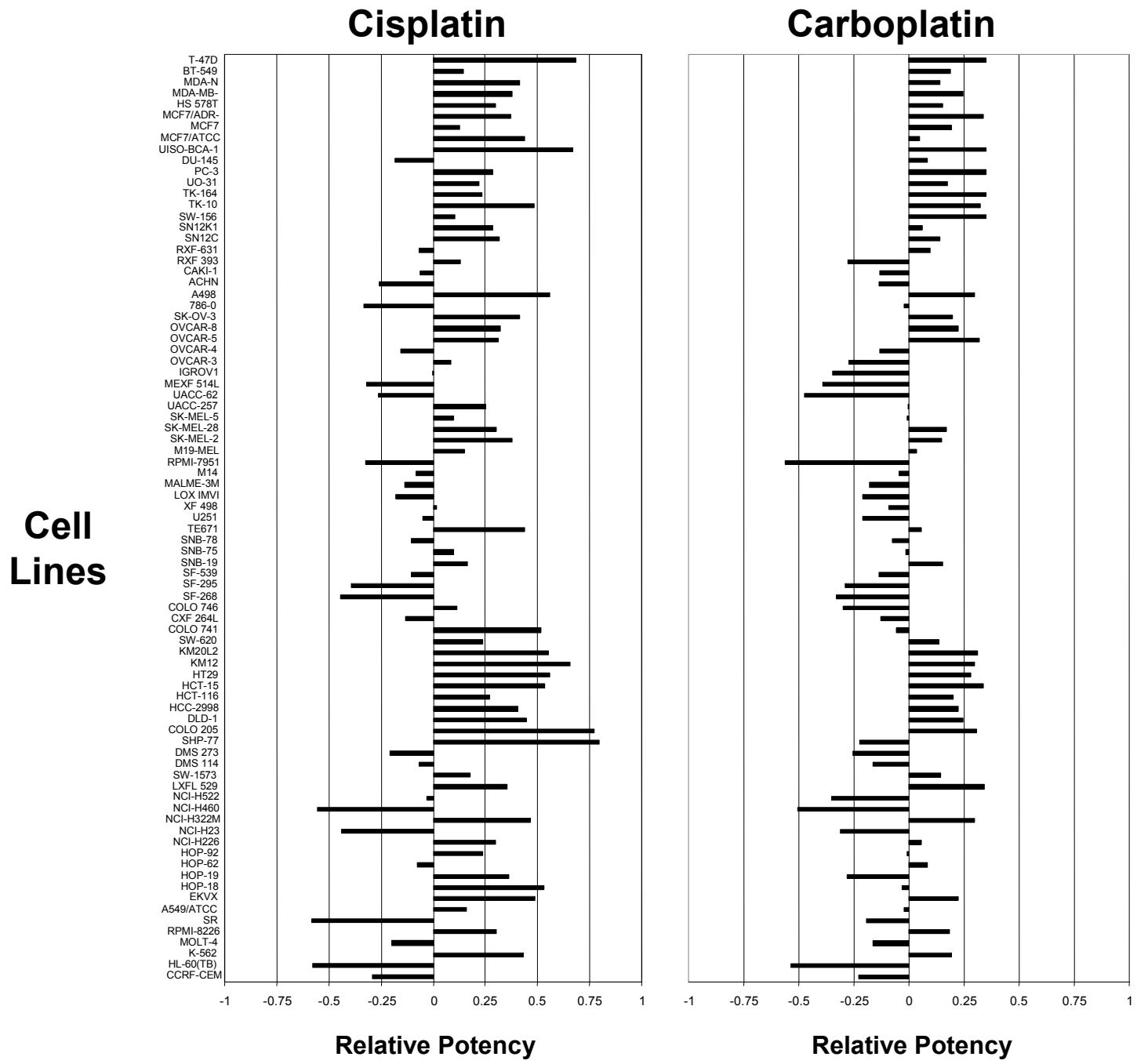
- Over 85% of compounds screened have no antiproliferative activity
- Beginning 1999 all compounds are screened against 3 highly sensitive cell lines
  - Breast MCF-7
  - Lung NCI-H640
  - Glioma SF-268
- Demonstration of growth activity required for advancement to 60 cell line, five dose testing

NCI 60 Cell Line Screen

- "Disease-oriented" philosophy implemented in 1985 to 1990
- 60 different human tumor lines
  - Original: brain, colon, leukemia, lung, melanoma, ovarian, renal
  - Later: breast and prostate
- Automated sulforhodamine blue cytotoxicity assay
- Relative potency of a compound against all 60 cell lines determined at 5 doses
  - GI_{50} concentration that inhibits growth by 50%
  - TGI concentration that totally inhibits growth
  - LC_{50} concentration that kills 50% of cells

COMPARE Analysis

- **Computerized analysis of relative sensitivity of the different cell lines can categorize active agents using the COMPARE program**
- **Can identify similar classes of agents (i.e., top1 or top2 inhibitors, platinum analogues, TS inhibitors, etc)**
- **Can identify novel agents with unique activity patterns**


Cisplatin
Carboplatin
Cell Lines
T-47D
BT-549
MDA-N
MDA-MB-
HS 578T
MCF7/ADR-
MCF7
MCF7/ATCC
UISO-BCA-1
DU-145
PC-3
UO-31
TK-164
TK-10
SW-156
SN12K1
SN12C
RXF-631
RXF 393
CAKI-1
ACHN
A498
786-0
SK-OV-3
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
MEXF 514L
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
M19-MEL
RPMI-7951
M14
MALME-3M
LOX IMVI
XF 498
U251
TE671
SNB-78
SNB-75
SNB-19
SF-539
SF-295
SF-268
COLO 746
CXF 264L
COLO 741
SW-620
KM20L2
KM12
HT29
HCT-15
HCT-116
HCC-2998
DLD-1
COLO 205
SHP-77
DMS 273
DMS 114
SW-1573
LXFL 529
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
HOP-19
HOP-18
EKVX
A549/ATCC
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1
Relative Potency
Relative Potency

Selection of Active Compounds

- **Significant average potency**
- **Novel pattern of activity in 60 cell lines using the COMPARE algorithm**
- **Special interest based on chemical structure or biologic activity**
- **Recommendation of advisory committees**

Current NCI Development Strategy

- **NCI Decision Network (DN) I**
- **NCI DN IIA**
- **NCI DN IIB**
- **NCI DN III**

NCI Decision Network I

- **Post-60 cell line screening point**
- **Determine if in vivo hollow fiber 12 cell line testing is indicated**
- **Determine if in vivo xenograft animal testing is indicated**
  - **Requires T/C < 50% (IP drug administration/SC tumor) for further development**

NCI Decision Network IIA

- **Determine an acceptable formulation**
- **Determine optimal dose, route, & schedule**
- **Procurement of sufficient amounts of drug**
- **Feasibility of clinical administration**
- **Pharmacokinetic assays development**

NCI Decision Network IIB

- **Substantial resource commitment**
- **GMP manufacturing**
- **Toxicology in 2 species (one non-rodent) with histopathological correlation**
- **Animal pharmacokinetic studies**

NCI Decision Network III

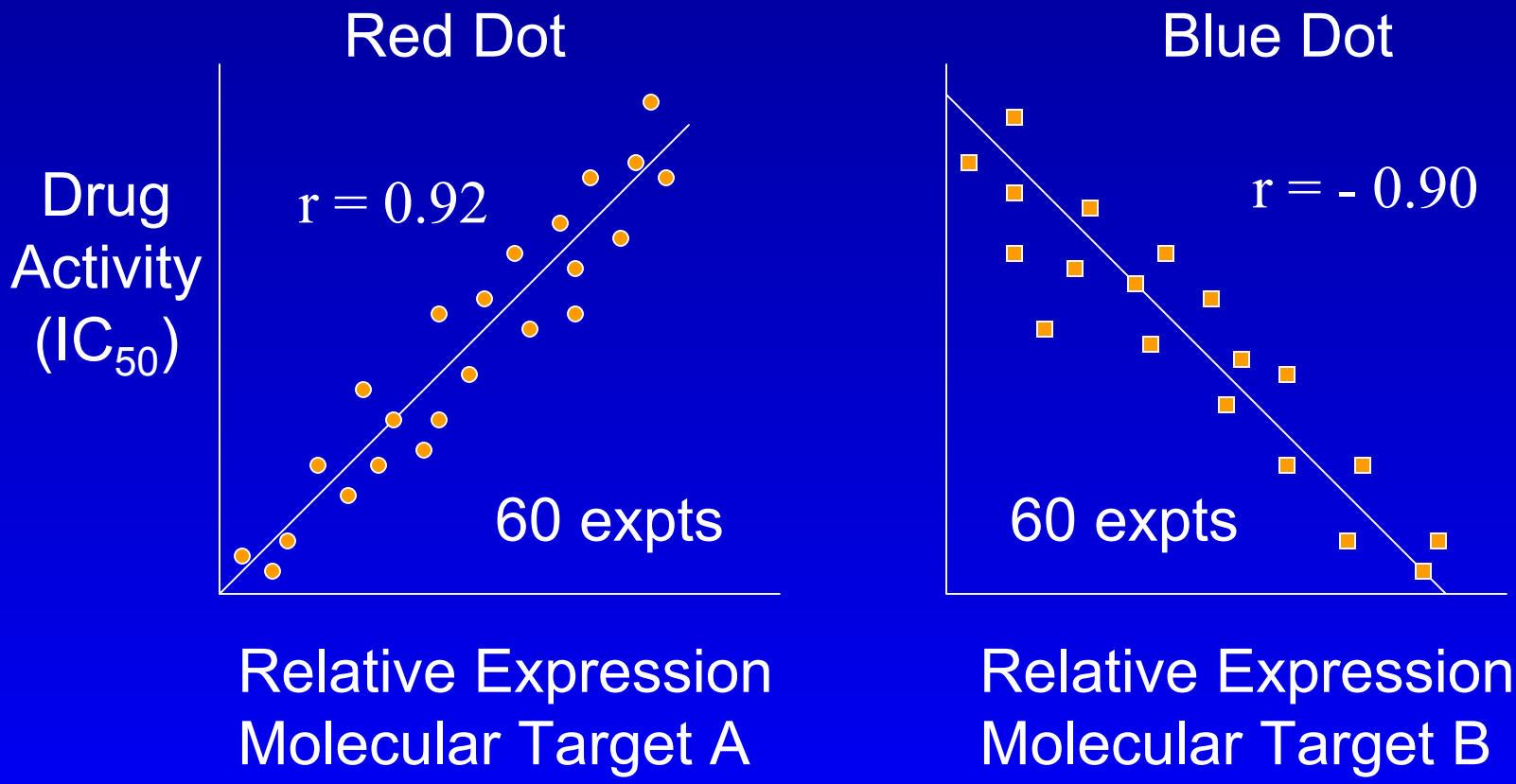
- **Planning for initial phase I trials**
- **Estimation of a safe starting dose**
- **NCI sponsored IND filed with FDA**
- **NCI funded clinical research program planned for phase I, phase II and possible phase III testing overseen by the Cancer Treatment Evaluation Program (CTEP)**

Targeted-Based Drug Discovery

- **Understanding the molecular defects that generate human tumors will identify new and novel targets for pharmacologic intervention**
- **Screening based upon molecular targets**
- **Yeast-based drug discovery**
- **NCI 60 cell line screen: molecular targets defined**

Target-Based Drug Screening

NCI 60 Cell Line Panel



Implications of Molecular Target-Based Screening

- **Compounds entering preclinical and clinical development will have specific targets defined for their mechanism of action**
- **Understanding these mechanism will be important for the design and conduct of early clinical trials of these agents**

How is the emphasis on molecular targeting changing the approach to preclinical and early clinical drug development?

EXAMPLE: STI571, A Molecularly Targeted Anticancer Agent

STI-571 (Imatinib Mesylate, Gleevec®)

- **1992: Ciba Geigy (Novartis) identified STI571 as a potent inhibitor of the platelet-derived growth factor receptor (PDGF-R)**
  - **Originally synthesized as an anti-inflammatory agent!**
- **1995-1996: STI571 recognized by Dr. Brian Druker at U. Oregon as a specific inhibitor targeting the Bcr-Abl tyrosine kinase in laboratory experiments**

c-abl Tyrosine Kinase

- **In 1970's the transforming retrovirus oncogene (*v-Abl*) was identified in the Abelson leukemia virus**
- **Cellular homologue, *c-Abl*, located on chromosome 9**
- **The *c-Abl* gene product (enzyme) transfers a phosphate group from ATP to tyrosine residues on target proteins**
- **Signals the cell to proliferate and grow**
  - **c-Abl is an accelerator switch, telling cells to grow**

Chronic Myelogenous Leukemia and the Ph Chromosome

- **Abnormal Philadelphia (Ph) chromosome identified in most patients with chronic myelogenous leukemia (CML)**
- **Piece of chromosome 9 is abnormally linked to chromosome 22**
  - **9:22 translocation**
- **Identified in over 90% of CML, 20% of adult ALL and 5% of pediatric ALL patients.**

Bcr-Abl Tyrosine Kinase

- **Translocation 9:22 abnormally links the c-Abl gene to the Bcr region on chromosome 22**
  - **Bcr = breakpoint cluster region**
- **Bcr-Abl fusion gene turns on the c-Abl tyrosine kinase**
- **Constitutive activation of Bcr-Abl signals the cell to proliferate in an uncontrolled fashion**
  - **Causes leukemic cells to proliferate in CML**
  - **c-Abl accelerator switch is stuck in the "on" position**

STI571 in CML: The Rationale

- **Dr. Brian Druker recognized if the Bcr-Abl accelerator is switched on in CML, then STI571 may be able to shut down its function**
- **Theoretically this should affect CML cells, but not normal cells**
  - **A selective "brake" on abnormal cell growth**
- **Laboratory studies with STI571 show marked growth inhibition of CML cells**

Phase I Studies with STI571

(Druker et al NEJM 2001)

- **Oral STI571 at 25 to 1000 mg/d administered to 83 patients with chronic phase CML**
- **Mild-moderate toxicities:**
  - **Nausea 43%**
  - **Myalgias 41%**
  - **Edema 39%**
  - **Diarrhea 25%**
  - **Reversible transaminitis 8%**
  - **Grade 3 thrombocytopenia 16%**
  - **Grade 3 neutropenia 14%**
- **No dose limiting toxicities seen!**

Phase I Studies with STI571

(Druker et al NEJM 2001)

- **In 54 patients treated with ≥300 mg/d of STI571**
  - **Hematological response in 53 (98%)**
    - **50% fall in WBC**
  - **Cytogenetic bone marrow responses in 29 (54%)**
    - **Partial response: 1-35% of BM cells Ph positive**
    - **Minor response: 35-65% of BM cells Ph positive**
  - **Complete cytogenetic remissions in 7 (13%)**
    - **No BM cells Ph positive**
- **96% of hematologic responders still in remission at 8.8 months follow up**

Clinical Trials with STI571

- **Phase II trials initiated in 1999 in CML showed high response rates even in patients in accelerated phase**
- **FDA approval of STI571 in 2001, 5 years after its anticancer activity was recognized in the laboratory**

STI571 in Solid Tumors

- **c-kit Tyrosine Kinase:**
  - **Gastrointestinal stromal tumors (GIST), mast cell leukemia, gem cell tumors, SCLC, neuroblastoma, melanoma, ovarian and breast cancers**
  - **Preliminary response rates of 53% in GIST phase II trials (approved by FDA in 2002)**
- **PDGF-R Tyrosine Kinase**
  - **Sarcomas, glioblastomas, non-small cell lung, breast, and prostate cancers**
- **Clinical trials in various solid tumors are ongoing**

Conclusions

- **Development of ST571 is the latest and best example of molecularly targeted therapy to date**
- **Illustrates a new strategic approach to developing cancer therapies in the "post-genomic" era**

What can we learn from the STI571 example?

Dawn of a New Era? Viewpoint 1: Yes!

- **STI571 paves the way for targeted cancer therapies**
- **A New Paradigm: Molecular phenotype determines response**
  - **Identify novel tumor targets/phenotype**
  - **Screen for agents that hit these targets**
  - **Take the most promising agents into clinical trials**
- **Conclusion: Hey, this is going to be easy!**

(C. Sawyers, ASCO 2002)

Dawn of a New Era? Viewpoint 2: No So Fast!

- **STI571 is a nice model, but it may be unique.**
- **CML and GIST are rare "single hit" cancers that are rarely invasive**
- **Most common solid tumors have multiple genetic abnormalities (>5)**
  - **Multiple inhibitors of each may be necessary**
- **Conclusion: Hey, this is too complex!**

(C. Sawyers, ASCO 2002)

Back to Reality

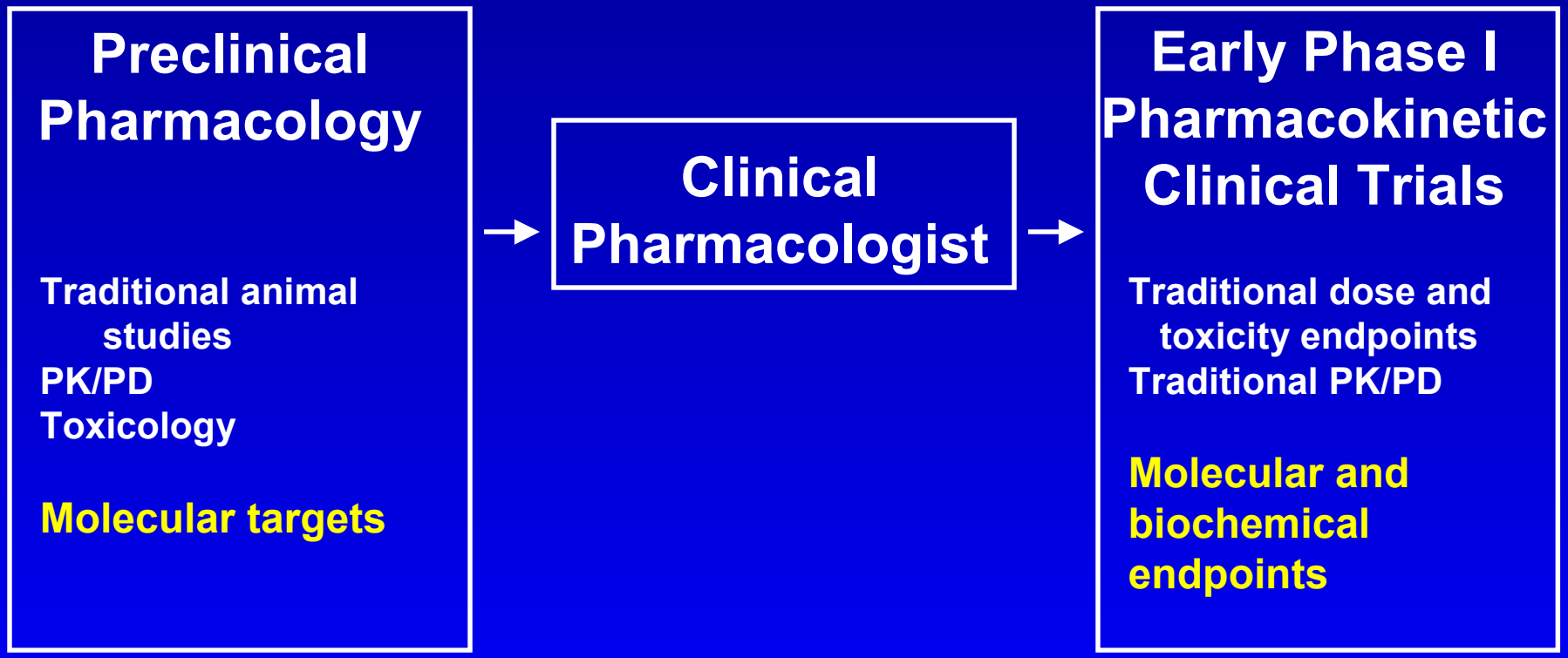
- **The truth lies is some where in between**
- **STI571 shows us the power and potential of understanding molecular targets in cancer cells**
- **The road is long, complex, but also filled with promise**

The Clinical Trial Challenge

- **We stand at the dawn of the post genomic era when new targets for novel treatments for human cancer are just being discovered and defined**
- **Basic research is the engine that drives this process**
- **Clinical researchers have to take these promising agents and test them in the best and most efficient ways possible**
  - **Traditional clinical endpoints, and…**
  - **Molecular target endpoints in clinical studies**

As clinical pharmacologists, how does molecular targeting affect the design and conduct of early clinical trials in drug development?

The Challenge!



New Paradigms for Drug Development

- **Expanding role for clinical pharmacologists**
- **To bridge the gap between preclinical pharmacologic studies and early clinical trials**
- **New molecular and biochemical endpoints are essential for cancer prevention and antimetastatic agents**

IDD Agents in Active Testing

2001

ABX-EGF
AVE8062A
BCH-4556
BEXAROTENE
BIZELESIN
BMS-184476
BMS-247550
BMS-247616
CCI-779
CI-1033
COL-3
DE-310
DJ-927
DX-8951f (exatecan)
ET-743
EKB 569
FB-642
FMdC
G3139
HMN-214
INGN
ING-I (HEMAB)
INTOPLICINE
LOMETREXOL
LY231514 (MTA)
LY355703
MGI 114
MSI-1256F
NX-211
OXALIPLATIN
OSI-774
PANOREX
PEG-Camptothecin
PEG-Paclitaxel
R115777
REBECCAMYCIN
RFS 2000
RPR 116258A
R115777
SB-408075
SR-45023A
T138067
TAZAROTENE
ZD1839 (Iressa)
ZD9331
ZD0473
STI-571
huKS-IL2